2606-Pos Board B298
Human Guanylate-Binding Protein 1 Tethers Giant Unilamellar Vesicles in a Nucleotide-Dependent Manner
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Human guanylate-binding protein 1 (hGBP1) is the most studied protein within the family of guanylate-binding proteins (GBPs), which has seven isoforms in humans. GBPs belong to the dynamin superfamily of large GTPases and are thought to act as mechanoenzymes. All members of the GPBs family are expressed to high level after treatment of the cells with interferons, and hGBP1 in particular is mostly expressed by interferon γ, and, similar to the family of Mx proteins, is involved in antiviral response. However, the molecular mechanism of antiviral activity of hGBP1 is poorly understood. In the course of posttranslational modification the protein is coupled to a lipid anchor (isoprenoid), which might be crucial for performing its function within the cell. We address the question of the molecular function of hGBP1 by studies of its farnesylated form in vitro in the presence and absence of lipid systems. We can show nucleotide-dependent polymerization of the farnesylated form of hGBP1 and, moreover, we can show that the non-farnesylated form of hGBP1 disturbs the latter processes giving a hypothesis of possible regulation of the biological function of the protein by other isoforms from GBPs family, which cannot undergo lipid modification, through the heterointeraction. Previous studies of protein interaction with lipids show the binding of the protein to the liposomes only in the active state of the protein. In contrast, by using the lipid model of giant unilamellar vesicles (GUV), we can show that the protein, which carries the farnesyl anchor, binds to the vesicles directly after nucleotide binding and does not require GTP hydrolysis. Also we can show that it tethers vesicles in a nucleotide-dependent manner and we assume this to be related to the biological function of the protein.

2607-Pos Board B299
Transmembrane Domains of Bacterial Cell Division Proteins FTSB and FTSL Form a Stable High-Order Oligomer - A Fret Study
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FtsB and FtsL are two essential integral membrane proteins of the bacterial division complex or "divisome", both characterized by a single transmembrane helix and a juxta-membrane coiled coil domain. The two domains are important for the association of FtsB and FtsL, a key event for their recruitment to the divisome, that in turn enables recruitment of the late divisomal components to the Z-ring and subsequent completion of the division process. Here we present a biophysical analysis performed in vitro that shows that the transmembrane domains of FtsB and FtsL associate strongly in isolation. Using FRET, we have measured the oligomerization of fluorophore-labeled transmembrane domains of FtsB and FtsL in both detergent and lipid. The data indicates that the transmembrane helices are likely a major contributor to the stability of the FtsB-FtsL complex. Our analyses show that FtsB and FtsL form a 1:1 higher-order oligomeric complex, possibly a tetramer. This finding suggests that the FtsB-FtsL complex is capable of multi-valent binding to FtsQ and other divisome components, a hypothesis that is consistent with the possibility that the FtsB-FtsL complex has a structural role in the stabilization of the Z-ring.

2608-Pos Board B300
General Anesthetics do not Alter Lipid Bilayer Properties at Clinically Relevant Concentrations
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General anesthetics are a widely used class of drugs but, despite their clinical use for >160 years, their exact molecular mechanism(s) remain to be elucidated. A mechanism proposed early on was direct interaction with the lipid bilayer, in some unspecified manner to alter cellular function, which lead to the unitary lipid-based hypothesis of anesthetic action. More recent studies show that general anesthetics interact specifically with various proteins, in particular membrane-embedded ion channels. For example, the inhibition of voltage-gated sodium channels by volatile anesthetics leads to reduced neurotransmitter release in excitable cells. But, though a number of anesthetic targets have been identified, it remains unclear whether the bilayer per se may be involved as well. We therefore examined whether various general anesthetics (isoflurane, sevoflurane, halothane, desflurane, chloroform, diethyl ether, F3, cyclopropane, ketamine and etomidate) and related nonanesthetics (F6 and flurothyl) alter lipid bilayer properties at clinically relevant concentrations. The effects on lipid bilayer properties were tested using the gramicidin-based fluorescence assay (GBFA). The results show that none of the anesthetics or nonanesthetics tested altered lipid bilayer properties at the clinical concentration of 1 MAC (minimal alveolar concentration) with a membrane mole-fraction ranging from 1x10^-3 (for F6) to 0.1 (for diethyl ether and sevoflurane). Even at two- to four-fold higher concentrations only minimal effects on the bilayer were observed; at much higher (supratherapeutic) concentrations, however, certain anesthetic agents did alter lipid bilayer properties. These results suggest that general anesthetics do not alter ion channel function by altering lipid bilayer properties in a manner that is sensed by a bilayer-spanning channel at clinically relevant concentrations.

2609-Pos Board B301
Arf1 Induced Membrane Remodeling and Morphological Changes Studied by Cryo-Em, Confocal Microscopy and Langmuir Film Balance
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The small GTPase Arf1 of the RAS superfamily plays an important role in vesicular trafficing. On the Golgi membrane, the formation and fission of coat protein I (COPI) transport vesicles proceeds via local deformation of the lipid bilayer by a curvature generating COPI protein coat. The assembly of this complex is initiated by the GTPase Arf1 in a nucleotide-dependent manner. After GDP/GTP exchange, soluble Arf1 becomes membrane bound by insertion of its myristoylated N-terminal amphipathic helix (myrAH) into the proximal leaflet of the Golgi membrane. The subsequent liberation of transport vesicles requires the full COPI complex and has been observed in vivo and in vitro.
As the role of Arf1 in the process of curvature induction has not been fully elucidated, we have studied binding and incorporation of recombinant S. cerevisiae Arf1p into lipid mono- and bilayers using binding assays with a Langmuir film balance setup and artificial, unilamellar liposomes. We observe myristoylation-dependent binding to membranes and an increase in membrane surface area upon addition of Arf1p. Confocal laser scanning microscopy and cryo electron microscopy reveal highly curved membrane structures upon incorporation of myristoylated Arf1p. Our results support a mechanism of curvature induction based on the bilayer couple theory.

2610-Pos Board B302
The Dynamics of P-Rex 2 Membrane and Protein Interactions
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P-Rex 2 (phosphatidylinositol (3,4,5)-trisphosphate Rac exchanger 2) protein is a Rac guanine nucleotide exchange factor (GEF) whose activity is tightly regulated through activation by PI(3,4,5)P3 (phosphatidylinositol 3,4,5-trisphosphate) and G-protein βγ subunits along with phosphorylation and domain-domain interactions. A previous study has shown that P-Rex 2 DH-PH domains interacts with PTEN (phosphate and tensin homologue deleted on chromosome 10), which is a frequently mutated tumor suppressor in human cancer that dephosphorylates PI(3,4,5)P3 and antagonizes PI3K signaling. P-Rex 2 binds to PTEN through its DH-PH domain, inhibits PTEN's lipid phosphatase activity and activates the PI3K pathway. This suggests that P-Rex 2 may function as a regulator of PTEN and activator of the PI3K pathway, thereby, contributing to a variety of pathological and physiological processes, such as tumorigenesis, diabetes, and aging. We are investigating the role of P-Rex 2 DH-PH domains in lipid binding and PTEN protein binding using steady-state fluorescence, stopped flow photometry, and ITC (isothermal titration calorimetry). This study aims to delineate P-Rex 2 membrane and protein interactions at the molecular level.

2611-Pos Board B303
The Effect of Hydrophobic Matching between Lipids and Transmembrane Peptides on Sterol Bilayer Affinity
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Lipid self-organization is believed to be essential for shaping the lateral structure of membranes, but it is becoming increasingly clear that also membrane proteins can be involved in the maintenance of membrane architecture. Cholesterol is thought to be important for the lateral organization of eukaryotic cell membranes and has also been implicated to take part in the sorting of cellular